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(54) Organic amine phosphonic acid complexes for the treatment of calcific tumors.

(57) Particle-emitting radionuclides, eg. Samarium-153, have been complexed with phosphonic acid derivatives of organic amine or substituted organic amine compounds wherein the nitrogen and phosphorus are interconnected by an alkylene group. These complexes have been found useful in the treatment of calcific tumors in animals.

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ORGANIC AMINE PHOSPHONIC ACID COMPLEXES
FOR THE TREATMENT OF CALCIFIC TUMORS

   Aminophosphonic acids are known to chelate
metal ions. Particularly stable chelates are formed
5 with metals from the alkaline earth and transition
metal series.

   The development of a bone metastasis is a
common and often catastrophic event for a cancer patient.
The pain, pathological fractures, frequent neurological
10 deficits and forced immobility caused by these metastatic
lesions significantly decreases the quality of life for
the cancer patient. The number of patients that contract
metastatic disease is large since nearly 50% of all
patients who contract breast, lung or prostate carcinoma
15 will eventually develop bone metastases. This indicates the
prevalence of the disease. Bone metastases are also
seen in patients with carcinoma of the kidney, thyroid,
bladder, cervix and other tumors, but collectively,
these represent less than 20% of patients who develop
20. bone metastases. Metastatic bone cancer is rarely life
threatening and occasionally patients live for years
following the discovery of the bone lesions. Initially,
treatment goals center on relieving pain, reducing
requirements for narcotic medication and increasing
25 ambulation. Clearly, it is hoped that some of the
cancers can be cured.

The use of radionuclides for treatment of
cancer metastatic to the bone dates back to the early
1950's. It has been proposed to inject a radioactive
particle-emitting nuclide in a suitable form for the
5   treatment of calcific lesions. It is desirable that
such nuclides be concentrated in the fast growing
portion of the bone with minimal amounts reaching the
soft tissue and normal bone. Radioactive phosphorus
compounds (P-32 and P-33) have been proposed. However,
10   the nuclear and biolocalization properties limit the
use of these compounds. (Kaplan, E., et al, Journal of
Nuclear Medicine, Vol. 1, No. 1, page 1, 1960); (U.S.
Patent 3,965,254).

Another attempt has been made using phosphorus
15   compounds containing a boron residue. The compounds
were injected into the body (intravenously) and accumu-
lated in the skeletal system. The patient was then
irradiated with neutrons in order to activate the boron
and give a therapeutic radiation dose. (U.S. Patent
20   4,399,817).

In the above-mentioned procedures, it is not
possible to give therapeutic doses to the tumor without
substantial damage to normal tissues. In many cases,
especially for metastic bone lesions, the tumor has
25   spread throughout the skeletal system and amputation or
irradiation of a portion of the body is not practical.
(Seminars in Nuclear Medicine, Vol. IX, No. 2, April,
1979).

The use of diphosphonate complexed with
30   Re-186 has also been proposed. (Mathieu, L. et al,
Int. J. Applied Rad. & Isotopes, Vol. 30, pp. 725-727,
1979; Weinenger, J., K tring, A. R., et al, Journal of

Nuclear Medicine, Vol. 24, No. 5, page 125, 1983).
However, the preparation and purification needed for
this complex limits its utility and wide application.

Strontium-89 has also been proposed for
5 patients with metastic bone lesions. However, the long
half-life (50.4 days), high blood levels and low lesion
to normal bone ratios limit the utility. (Firusian,
N., Mellin, P., Schmidt, C. G., The Journal of Urology,
Vol. 116, page 764, 1976; Schmidt, C. G., Firusian, N.,
10 Int. J. Clin. Pharmacol., 93:199-205, 1974).

A palliative treatment of bone metastases has
been reported which employed I-131 labelled α-amino-(3-
iodo-4-hydroxybenzylidene)diphosphonate (Eisenhut, M.,
Journal of Nuclear Medicine, Vol. 25, No. 12, pp. 1356-
15 1361, 1984). The use of radioiodine as a therapeutic
radionuclide is less than desirable due to the well
known tendency of iodide to localize in the thyroid.
Eisenhut lists iodide as one of the possible metabolites
of this compound. In addition, any I-131 left over
20 from the iodination reaction and not separated in the
washing procedure also constitutes a threat to the
thyroid. Indeed Eisenhut finds 0.1% of the injected
dose present in the thyroid 24 hours after injection.
The high energy of the gamma ray emission from I-131
25 leads to inferior quality images.

Therapeutically useful complexes according to
the present invention must fit certain criteria insofar
as possible. It should be recognized that there are
many ligands, or complexing agents, which are included
30 by our definition of the aminophosphonic acids in which
the nitrogen of the amine and the phosphorus of the

phosphonic acid group are separated by an alkylene group. Many may also contain other functional groups as substituents for some, but not all, of the amine hydrogens of the ligand. It should also be recognized

5   that the properties of the particular isotope are important. The disadvantage of any one property may be overcome by the superiority of one or more of the properties of either ligand or isotope and their combination as the complex must be considered in toto.

10   The following is a discussion of the criteria which must be considered in choosing any particular combination of radioisotope and ligand.

         Firstly, the complex must be taken up preferentially by the bone rather than by soft tissue. Most

15   particularly, uptake in neither liver nor bone marrow is desired. Uptake by muscle tissue, while not as damaging, is also undesirable, resulting in "inferior" imaging properties as well as unwanted dosage to non-target organs.

20   Another important criterion is the ratio of the amount of complex taken up by the cancerous bone to that by normal bone. High ratios are preferred since it is desired to treat the cancerous bone while irradiating the normal bone as little as possible.

25   The complex should be cleared from the blood rapidly for obvious reasons.

         With respect to the complexed radionuclide, considerations of the nuclear properties are essential. First, the half-life must be sufficiently long to allow

30   for localization in the bone without delivering exc ssive

doses to non-target organs. When the complex is able to biolocalize rapidly, isotopes having shorter half-lives are useful. Isotopes with shorter half-lives provide an advantage since this permits the total dose

5   to be given fractionally, allowing any non-target organs damaged by the radiation to recover between doses.

While for successful treatment of tumors the isotope must have sufficient particle emission, it is

10   desirable, in order to quantify the localization of the complex, that the isotope also have sufficient gamma ray production that imaging is possible. The preferred gamma ray energy should be in the 100 to 200 keV (16 x 10^{-15} to 32 x 10^{-15} joule) range although some isotopes

15   having higher energies are potentially useful. The amount of gamma radiation should be sufficient to image, but not so great that the patient needs to be isolated to prevent him from becoming a source of radiation exposure to persons near him.

20   There is a need, therefore, for a system possessing the above criteria by which it is possible to deliver therapeutic radiation doses to calcific tumors with minimal doses to soft tissue or normal bone.

25   Such a system has now been found wherein a radioactive metal ion is complexed to a phosphorus-containing ligand. Certain of these complexes have been shown to be very selective for the skeletal system with very low soft tissue uptake. The material not

30   taken up by bone is efficiently cleared through the kidneys into the bladder. The complexes also tend to

concentrate in ar as of fast-growing bone much more
readily than in normal bon . The radionuclides used
ar particle-emitting and a high radiation dose is
delivered in the area where they are deposited. Thus,
5   therapeutic radiation doses can be delivered speci-
fically to calcific tumors.

Particle-emitting radionuclides, e.g. Sama-
rium-153, Yb-175, Lu-177 and Gd-159, have been complexed
with organic amine or substituted organic amine phosphonic
10   acid derivatives wherein the nitrogen and phosphorus
are interconnected by an alkylene or substituted alky-
lene group. These complexes have been found useful in
the treatment of calcific tumors in animals.

The proposed use for the complexes of this
15   invention is the therapeutic treatment of calcific.
tumors. These include primary tumors, where the skeletal
system is the first site of involvement, and metastic
bone cancer where the neoplasm spreads from other
primary sites, e.g. prostate, breast, into the skeletal
20   system. This invention provides a method of alleviating
pain by delivering a therapeutic radiation dose to the
aforementioned calcific tumors. This invention also
provides a means of reducing the size of or destroying
the calcific tumors by delivering a therapeutic radiation
25   dose.

The organic phosphonic acid derivatives which
have been found useful in complexing the particle-emitting
radionuclides are organic amine or substituted organic
amine compounds wherein the nitrogen and phosphorus are
30   interconnected by an alkylene or substituted alkylene
group having the formula

$$-\!\!\left(\!\!\begin{array}{c} X \\ | \\ C \\ | \\ Y \end{array}\!\!\right)_{\!\!n}\!\!-$$
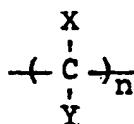
wherein X, Y are independently selected from hydrogen, hydroxyl, carboxyl, phosphonic, and hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable salts of the acid radicals and n is 1-3 with the proviso that when $n > 1$, each X and Y may be the same as or different from the X and Y of any other carbon atom.

The compounds can be prepared by a number of known synthetic techniques. Of particular importance is the reaction of a compound containing at least one reactive N-H group with a carbonyl compound (aldehyde or ketone) and phosphorous acid or derivative thereof.

The following structural formulas represent some of the complexing ligands which can be used in the treatment of calcific tumors when complexed with a particle-emitting radionuclide:

$$\begin{array}{ccc} A & & B \\ & \diagdown \!\! N \!\! \diagup & \\ & | & \\ & C & \end{array}$$

and

$$\begin{array}{c} A\diagdown \qquad \qquad \qquad \diagup C \\ \diagup \!\! N\!\!-\!\!(R\!-\!N)_{\overline{m}}R\!-\!N\diagdown \\ B \qquad \quad | \qquad \qquad \diagdown D \\ (R\!-\!N)_{\overline{m'}} E \\ | \\ F \end{array}$$
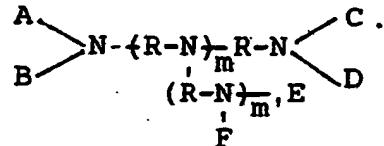
wherein substituents A, B, C, D, E and F are independently selected from hydrogen, $-\!\!\left(\!\!\begin{array}{c} X \\ | \\ C \\ | \\ Y \end{array}\!\!\right)_{\!\!n}\!\!OH$, $-\!\!\left(\!\!\begin{array}{c} X \\ | \\ C \\ | \\ Y \end{array}\!\!\right)_{\!\!n}\!\!COOH$, $-\!\!\left(\!\!\begin{array}{c} X \\ | \\ C \\ | \\ Y \end{array}\!\!\right)_{\!\!n}\!\!PO_3H_2$,

and physiologically acceptable salts of the acid radicals wherein X, Y, and n have been previously defined and m and m' each is 0-10 with the proviso that at least one of said nitrogen substituents is a phosphorus-containing

group and wherein R is a hydrocarbon residue which can
be a lin ar, branched, cyclic, heterocyclic, substituted
heterocyclic, or a fused ring-type structure; with the
further proviso that when m or m' >1 the E and F substi-
5   tuents may be the same as or different from any other
substituent of any other nitrogen atom and each R can
be the same as or different from any other R.

Methods for carboxyalkylating to obtain the
amine derivatives containing one or more carboxyalkyl
10   groups are well known (U.S. 3,726,912) as are the
methods which give alkyl phosphonic and hydroxyalkyl
(U.S. 3,398,198) substituents on the amine nitrogens.

Some specific, but non-limiting, examples of
compounds which are included by the above structures
15   are ethylenediaminetetramethylenephosphonic acid (EDTMP),
diethylenetriaminepentamethylenephosphonic acid (DTPMP),
hydroxyethylethylenediaminetrimethylenephosphonic acid
(HEEDTMP), nitrilotrimethylenephosphonic acid (NTMP) and
tris(2-aminoethyl)aminehexamethylenephosphonic acid
20   (TTHMP).

Examples of particle-emitting nuclides useful
in the practice of the invention are Sm-153, Yb-175,
Lu-177 and Gd-159.

While Sm-153, Yb-175, Lu-177 and Gol-159 have
25   been exemplified as the rare earth radionuclides
employed with the complexing agents above, other rare
earth radionuclides may also be complexed with the same
aminophosphonic acid derivatives as disclosed herein.
Representative, but non-limiting, example of such rare
30   earth radionuclide is Ho-166.

The pr ferred embodiment of the present invention
is a therapeutically effective compl x of a particle-
emitting radionuclide selected from the group consisting
of gadolinium-159 (Gd-159), holmium-166 (Ho-166),
lutetium-177 (Lu-177), samarium-153 (Sm-153) and ytter-
bium-175 (Yb-175) with an aminophosphonic acid derivative
selected from the group consisting of ethylenediaminetetra-
methylenephosphonic acid (EDTMP), diethylenetriaminepenta-
methylenephosphonic acid (DTPMP), hydroxyethylethylenedi-
aminetrimethylenephosphonic acid (HEEDTMP), nitrilotri-
methylenephosphonic acid (NTMP) and tris(2-aminoethyl)amine-
hexamethylenephosphonic acid (TTHMP).

For the purpose of convenience the abbreviations
given in the parentheses above will be used to denote
the respective radionuclides and aminophosphonic acid
derivatives hereinafter.

A more preferred embodiment of the present
invention is a therapeutically effective complex of a
particle-emitting radionuclide selected from the
group consisting of Gd-159, Lu-177, Sm-153, and Yb-175
with an aminophosphonic acid derivative selected from
the group consisting of EDTMP, DTPMP, HEEDTMP, NTMP and
TTHMP.

Particularly preferred embodiment of the
present invention is a therapeutically effective complex
of the particle-emitting radionuclide selected from
the group consisting of Lu-177, Sm-153 and Yb-175 with
an aminophosphonic acid derivative selected from the
group consisting of EDTMP, DTPMP, HEEDTMP, NTMP and
TTHMP.

The most preferr d embodiment of the pr sent
inv ntion is a therap utically effectiv complex of
Sm-153 with an aminophosphonic acid d rivative selected
from the group consisting of EDTMP, DTPMP, HEEDTMP,
5   and TTHMP.

For the purpose of the present invention,
therapeutically effective complexes described herein
and physiologically-acceptable salts thereof are con-
sidered equivalent. Physiologically-acceptable salts
10   refer to the acid addition salts of those bases which
will form a salt with at least one acid group of the
complex and which will not cause an adverse physio-
logical effect when administered to an animal at
dosages consistent with good pharmacological activity.
15   Suitable bases include, for example, the alkali metal
and alkaline earth metal hydroxides, carbonates, and
bicarbonates such as sodium hydroxide, potassium hydroxid ,
calcium hydroxide, potassium carbonate, sodium bicarbonate,
magnesium carbonate and the like, ammonia, primary,
20   secondary and tertiary amines and the like. Physiologi-
cally-acceptable salts of the present invention may be
prepared by treating the complex having at least one
acid group with an appropriate base.

Radionuclides can be produced in several
25   ways. In a reactor, a nuclide is bombarded with neutrons
to obtain a nuclide with additional neutrons in its
nucleus.

e.g. Sm-152 + neutron ——————→ Sm-153 + gamma

Another method of obtaining radioisotopes is
by bombarding nuclides with linear accelerator or
cyclotron-produced particles. Yet another way of
obtaining radioisotopes is to isolate them from fission
5 product mixtures. The method of obtaining the radio-
nuclide is not critical to the present invention.

When aqueous solutions of metal ions are
mixed with solutions containing complexing agents, such
as those described in this invention, a complex between
10 the metal ion and the ligand can be formed as shown by
the equation below.

$$M + L \rightleftharpoons M \cdot L$$

The reaction is believed to be an equilibrium
such that the concentrations of metal (M) and complexing
15 agent (L) can affect the concentration of species
present in solution. Competing side reactions, such as
metal hydroxide formation, can also occur in aqueous
solution.

$$xM + yOH^- \longrightarrow M_x(OH)_y$$
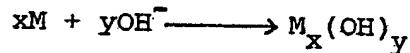
20 The OH^- concentration in solution which is
related to pH is, therefore, an important parameter to
be considered. If the pH is too high, the metal tends
to form metal hydroxides rather than complexes. The
complexing agents may also be affected by low pH.
25 Complexation may require the loss of protons; therefore
at low pH, conditions may not be favorable for complex-
ation to occur. The complexes of this invention can be
formed at several pH's. Consideration must be given to
the solubility characteristics of the ligand, radionuclide,

and compl x. Although complexation will occur at other
pH values, pH from 5-11 is preferred for complexation.

The metal and ligand may be combined under
any conditions which allow the two to form a complex.
5   Generally, mixing in water at a controlled pH (the
choice of pH is dependent upon the choice of ligand and
metal) is all that is required. In some cases heating
may be required to obtain maximum complex yield.

The ratio of ligand to metal is a result of
10   two competing considerations. As indicated above the
ligand and metal are believed to be in equilibrium with
the complex. On the one hand, it is desirable to have
a large quantity of ligand (L), so that there is a
minimum amount of free metal (M), because the uncom-
15   plexed metal may cause serious side effects in the
patient. On the other hand, excess free ligand may
compete for sites in the target, thus rendering the
treatment less effective. The fact that the ligands
within the scope of the invention are capable of complex-
20   ing with varying molar ratios of metal adds a degree of
complexity to these considerations. Although it is
difficult to generalize, it is believed that the ligand
to metal molar ratio is desirably 0.1:1 to 3000:1,
preferably 1:1 to 2000:1, more preferably 1:1 to 1000:1.

25   The samarium used in the following example
was either natural Sm_2O_3 (99.9 percent from Spex Indus-
tries) or isotopically enriched (99.06 percent Sm-152)
Sm_2O_3.

The Sm-153 used in this study was produced by
30   neutron irradiation at the University of Missouri

Research Reactor. Preliminary studies were carried out using Sm-153 produced by short (5-30 minutes) irradiations of natural Sm_2O_3, in the r actor's pneumatic tube system. The specific activity of Sm-153 produced by

5 this method was 0.5-3.0 Ci/g (18.5 to 111 GBq/g).

The majority of this work was carried out using Sm-153 produced by irradiating 99.06 percent enriched $^{152}Sm_2O_3$ in the first row reflector at a neutron flux of 1 x 10^{14} neutron/cm^2·sec. Irradiations

10 were generally carried out for 50-60 hours, yielding a Sm-153 specific activity of 1000 - 1300 Ci/g (37 x 10^3 to 48.1 x 10^3 GBq/g).

To irradiate Sm_2O_3 for production of Sm-153, the desired amount of target was first weighed into a

15 quartz vial, the vial flame sealed under vacuum and welded into an aluminum can. The can was irradiated for the desired length of time, cooled for several hours and opened remotely in a hot cell. The quartz vial was removed and transferred to a glove box, crushed

20 into a glass vial which was then sealed with a rubber septum and an aluminum crimp cap. One milliliter of 1-4 M HCl was then added to the vial via syringe to dissolve the Sm_2O_3. Once dissolved, the solution was diluted to the appropriate volume by addition of water.

25 The solution was removed from the original dissolution vial which contains the chards of the crushed quartz vial, and transferred via syringe to a clean glass serum vial. This solution was then used for complex preparation. Similar procedures were used to prepare

30 other radionuclides, e.g. Lu-177, Yb-175, Gd-159.

The various complex s employed in this invention wer prepared as follows: the desir d amount of ligand was placed in a vial and dissolved by addition of water. At some higher ligand concentrations, it was

5 necessary to add base in order to completely dissolve the ligand. Heating was also found to be useful for dissolving the ligands. The appropriate amount of the samarium or other radionuclides in the stock solution described above was then added to the ligand solution.

10 The pH of the resulting solution was then raised to the appropriate level by addition of NaOH. The solution was heated to 60°-70°C for 30 minutes in a water bath to insure maximum complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

15 The complex yield was determined by placing 5-20 microliters (depending on activity) of the complex solution onto a 0.5 cm³ column of a commercially available synthetic organic cation exchange resin. The column was then eluted with two separate 10 milliliter

20 volumes of isotonic saline. The anionic complexes are not retained by the resin and were eluted by the saline solution, while any uncomplexed metal was retained on the column. The eluant solutions and the column were then counted for the characteristic emission of the

25 particular radionuclide, e.g. 103 keV (16.5 x 10^{-15} joule) gamma ray of Sm-153. The complex yield was obtained by adding the counts in the eluant solutions and dividing by the total of the eluant solutions and the column.

30 This invention provides a means of delivering a therapeutic radiation dose to calcific tumors. It may also be desirable in the cases where the radionuclide has imageable gamma photons to inject a "sub-therapeutic"

dose and determine the fate of the radionuclide using a
scintillation camera prior to injecting a therapeutic
radiation dose. Ther fore, the levels of radiation
injected could be as low as 1 mCi (37 MBq) for imaging
5   prior to the therapy. Therapeutic doses will be greater.
The dose to the tumor may range from 100 to 10,000 rads
(1 Gy to 100 Gy). The preferred dose to the tumor
ranges from 1,000 to 8,000 rads (10 Gy to 80 Gy). For
complexes such as Sm-153-EDTMP amounts ranging from
10   0.1 mCi/kg body weight to 3 mCi/kg body weight are
preferred. The amount of activity required to deliver
a therapeutic dose may vary with the individual
radionuclides. Individual doses may be given in one
injection or fractionated into several injections
15   totaling the aforementioned dose per treatment.

       Biodistribution of various complexes of the
present invention was studied in rats and rabbits.

       Studies to determine the qualitative dis-
-tribution of the various complexes of the present
20   invention were conducted by injecting the complexes
into rats and obtaining the gamma ray images of the
entire animal at various times up to two hours after
injection.

       Male Spague Dawley rats were anesthetized by
25   injection with sodium pentabarbitol and the jugular
vein cannulated. The animals were then injected with
50 to 150 microliters of the various complexes and
gamma ray images were taken on a Nuclear Chicago®
Pho-Gamma® II scintillation camera immediately after
30   injection and approximately every half hour thereafter
for two hours.

Quantitative biodistributions wer obtained
by injecting 50-100 microlit rs of the complex solution
into the tail vein of unanesthetized male Spague Dawley
rats. The rats were then placed in cages lined with
5   absorbent paper in order to collect all urine excreted
prior to sacrifice. After two hours, the rats were
sacrificed by cervical dislocation and the various
tissues dissected out. The samples were then rinsed
with saline, blotted dry on absorbent paper and weighed.
10   The samples were counted with a NaI uptake counter
approximately one foot from the face of the crystal,
in order to minimize geometry differences between the
different size samples.

To minimize differences in age between groups
15   of animals, all rats used were in the 160-220 gram
weight range. All animals were kept "in-house" for
at least one week prior to use to minimize the effects
of stress that occur during shipping.

The various complexes of the present invention
20   were also evaluated in rabbits. The animals used in
these studies were male New Zealand white rabbits
in the 2.6 to 3.2 kilogram weight range. The animals
were kept "in-house" for at least a week prior to use.

The rabbits were injected with 100-250 micro-
25   liters of the complex solution via a cannula placed
in the marginal ear vein. In studies where blood
clearance was measured, blood samples were taken
through a heparinized cannula placed in the marginal
vein of the ear not used for injection of the complex.

30   Three hours after injection, a blood sample
was taken by cardiac puncture and the animal was then

sacrificed by injection of a commercial euthanasia solution. After sacrific , images were obtained by placing the carcass directly on the face of a large field of view scintillation camera.

5   Uptake of the complexes in the lesion compared to uptake in normal bone, or lesion/normal bone ratios, is a particularly important parameter for calculating the complex suitability as a therapeutic agent. To determine the lesion to normal bone ratios, a
10   modified drill hole method (Subramanian, G. et al., 19th lit. Annual Meetings of S.N.M., Bern, Switzerland, September 8-11, 1981) was used.

       In order to simulate uptake in rapidly growing bone, such as that found in cancerous bone, two
15   holes were drilled into the surface of the tibia of a rabbit in order to damage the bone. Seven to ten days later, the animal was injected with the complex. After three hours, the animal was anesthetized and imaged using an Anger camera and pinhole collimator.

20   The following examples are illustrative of the present invention and are not to be construed as limiting the scope thereof.

Example 1

       Into a suitable reaction vessel equipped with
25   a thermometer, magnetic stirring bar, dropping funnel, and an atmosphere of nitrogen were charged phosphorous acid (94.5 g) and degassed water (100 ml). Dissolution of the phosphorous acid was achieved by stirring and the solution was then treated with concentrated hydro-
30   chloric acid (112 ml). The dropping funnel was charged

with ethylenediamine (15 g) and adjusted to allow
dropwise addition of th diamine to the acidic solution.
When addition was compl te a heating mantle was installed
and the solution refluxed for one hour. At the end of
5   this time the dropping funnel was charged with formal-
dehyde (85 g of a 37% aqueous solution) which was add d
dropwise over a two-hour period with continued heating
to maintain reflux during the addition. After all th
formaldehyde was added, the reaction mixture was stirred
10 under reflux for an additional two hours, then allowed
to cool slowly overnight during which time the product
precipitated. Vacuum filtration followed by cold water
washing yielded   ethylenediaminetetramethylenephos-
phonic acid (EDTMP).

15. Example 2
        25 to 35 milligrams of EDTMP prepared in
Example 1 was weighed into a vial and dissolved using
0.75 ml of distilled water. To this, 0.25 ml of Sm-153
(~ 10 mCi(~ 370 MBq) in dilute HCl was added. The pH
20 of the resulting solution was then adjusted to 10 by
addition of NaOH. The resulting solution was heated to
60°C - 70°C for 30 minutes in a water bath to ensure
maximum complex formation. The pH of the solution was
then adjusted to 7-8 by addition of 1 M HCl. The yield
25 of the complex was over 95 percent.

        Laboratory rats were injected with the above
complex (50-100 $\mu\ell$) via the tail vein. After 2 hours,
the animals were sacrificed by cervical dislocation and
organs and tissues removed. Samples were counted with
30 a NaI uptake counter to determine the biolocalization
of the complex. It was found that a significant amount
(55-65 percent) of the activity was concentrated in the

skeletal system with very little soft tissue uptake.
Most of th activity not found in the skeleton was
cleared through the kidneys into the bladder. Scintil-
lation scans of animals treated in the same manner

5 showed the activity concentrating in the skeletal
system. The lesion to normal bone ratio (drill hole
model) (This data was obtained by the method of Subra-
manian, G., McAfee, J. G., et al, 19th Int. Annual
Meeting of S.N.M., Bern, Switzerland, September 8-11,

10 1981.) for this complex was approximately equal to that
of Tc-99m-MDP (MDP refers to methylene diphosphonate), a
commercially available diagnostic bone agent.

Example 3

Into a suitable reaction vessel equipped with

15 a thermometer, magnetic stirring bar, dropping funnel,
and an atmosphere of nitrogen were charged phosphorous
acid (94.5g) and degassed water (100 ml). Dissolution
of the phosphorous acid was achieved by stirring and
the solution was then treated with concentrated hydro-

20 chloric acid (112 ml). The dropping funnel was charged
with diethylenetriamine (20.6 g) and adjusted to allow
dropwise addition of the amine to the acidic solution.
When addition was complete a heating mantle was installed
and the solution refluxed for one hour. At the end of

25 this time the dropping funnel was charged with formal-
dehyde (85 g of a 37% aqueous solution) which was added
dropwise over a two-hour period with continued heating
to maintain reflux during the addition. After all the
formaldehyde was added, the reaction mixture was stirred

30 under reflux for an additional two hours, then allowed
to cool. Diethylenetriaminepentamethylenephosphonic acid
(DTPMP) was isolated from the reaction mixture.

Example 4

    20 to 30 milligrams of DTPMP prepared in
Example 3 was weighed into a vial and dissolved using
0.75 ml of distilled water. To this, 0.25 ml of Sm-153
5   (~ 10 mCi(~ 370 MBq)) in dilute HCl was added. The pH
of the resulting solution was then adjusted to 10 by
addition of NaOH. The solution was then heated to
60°C-70°C for 30 minutes in a water bath to ensure
maximum complex formation.

10   The pH of the solution was then adjusted to
7-8 by addition of 1 M HCl. The yield of the complex
was over 95 percent.

    The above complex was tested in rats. The
results in rats showed ~ 30 percent of the activity in
15 the skeletal system with very little activity in any
soft tissue.

Example 5

    Into a suitable reaction vessel equipped with
a thermometer, magnetic stirring bar, dropping funnel,
20 and an atmosphere of nitrogen were charged phosphorous
acid (94.5 g) and degassed water (100 ml). Dissolution
of the phosphorous acid was achieved by stirring and
the solution was then treated with concentrated hydro-
chloric acid (112 ml). The dropping funnel was charged
25 with N-hydroxyethylethylenediamine (34.6 g) and adjusted
to allow dropwise addition of the diamine to the acidic
solution. When addition was complete a heating mantle
was installed and the solution refluxed for one hour.
At the end of this time the dropping funnel was charged
30 with formaldehyde (85 g of a 37% aqueous solution)
which was added dropwise over a two hour period with

continued heating to maintain reflux during the addition. After all the formaldehyde was added, the reaction mixtur was stirred under reflux for an additional two hours, then allowed to cool. Hydroxyethylethylenediamine-
5   trimethylenephosphonic acid (HEEDTMP) was isolated from the reaction mixture.

Example 6

30 to 40 milligrams of HEEDTMP prepared in Example 5 was weighed into a vial and dissolved using
10 0.75 ml of distilled water. To this, 0.25 ml of Sm-153 (~ 10 mCi(~ 370 MBq)) in dilute HCl was added. The pH of the resulting solution was then adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C for 30 minutes in a water bath to ensure
15 maximum complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl. The yield of the complex was over 95 percent.

Example 7

Into a suitable reaction vessel equipped with
20 a thermometer, magnetic stirring bar, dropping funnel, and an atmosphere of nitrogen were charged phosphorous acid (57.7 g) and degassed water (50 ml). Dissolution of the phosphorous acid was achieved by stirring and the solution was then treated with concentrated hydrochloric
25 acid (50 ml). The dropping funnel was charged with tris(2-aminoethyl)amine (13.7 g) and adjusted to allow dropwise addition of the amine to the acidic solution. When addition was complete a heating mantle was installed and the solution refluxed for one hour. At the end of
30 this time the dropping funnel was charged with formalde-hyde (51 g of a 37% aqueous solution) which was added dropwise over a two-hour period with continued heating

to maintain reflux during the addition. After all the formaldehyde was added, the reaction mixture was stirred under reflux for an additional two hours, then allowed to cool. Tris(2-aminoethyl)aminehexamethylenephosphonic
5   acid (TTHMP) was isolated from the reaction.

Example 8

48 to 53 milligrams of TTHMP prepared in Example 7 was weighed into a vial and dissolved using 0.75 ml of distilled water. To this solution, 0.25 ml
10   of Sm-153 (~10 mCi(~ 370 MBq)) in dilute HCl was added. The pH of the resulting solution was adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C for 30 minutes in a water bath to ensure maximum complex formation. The pH of the solution was
15   then adjusted to 7-8 by addition of 1 M HCl.

The complexes of Examples 2, 4, 6 and 8 were tested in rats. The two-hour rat biodistribution data for these complexes is shown in Table I.

TABLE I

		Example Nos.		
		2	4	6	8
5	% Dose in Skeleton*	58 ±4	30 ±10	57 ±7	28 ±4
	Blood	0.032 ±0.016	0.16 ±0.12	0.035 ±0.006	0.25 ±0.14
	Liver	0.25 ±0.04	0.27 ±0.13	0.45 ±0.07	0.18 ±0.02
10	Urine	49 ±4	74 ±8	50 ±4	65 ±9
	Bone/Blood	1800 ±1200	224 ±150	1300 ±160	80 ±20
15	Bone/Muscle	1500 ±500	220 ±130	1300 ±180	410 ±75

Number of rats tested = 5

*Based on % dose in femur X 25

Example 9

20   35 to 45 milligrams of EDTMP prepared according to Example 1 was weighed into a vial and dissolved using 0.75 ml of distilled water. To this solution, 0.25 ml of Yb-175 in dilute HCl was added. The pH of the resulting solution was adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 25   70°C for 30 minutes in a water bath to ensure maximum complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

Example 10

55 to 60 milligrams of DTPMP prepared according 30   to Example 3 was weighed into a vial and dissolved

using 0.75 ml of distilled water. To this solution,
0.25 ml of Yb-175 in dilute HCl was added. The pH of
the resulting solution was adjusted to 10 by addition
of NaOH. The solution was then heated between 60°C and
5   70°C for 30 minutes in a water bath to ensure maximum
complex formation. The pH of the solution was then
adjusted to 7-8 by addition of 1 M HCl.

Example 11

        50 to 55 milligrams of HEEDTMP prepared
10 according to Example 5 was weighed into a vial and
dissolved with 0.75 ml of distilled water. To this
solution, 0.25 ml of Yb-175 in dilute HCl was added.
The pH of the resulting solution was adjusted to 10 by
addition of NaOH. The solution was then heated between
15 60°C and 70°C for 30 minutes in a water bath to ensure
maximum complex formation. The pH of the solution was
then adjusted to 7-8 by addition of 1 M HCl.

Example 12

        Into a suitable reaction vessel equipped with
20 a thermometer, magnetic stirring bar, dropping funnel,
and an atmosphere of nitrogen were charged phosphorous
acid (94.5 g) and degassed water (100 ml). Dissolution
of the phosphorous acid was achieved by stirring and
the solution was then treated with concentrated hydro-
25 -chloric acid (112 ml). The dropping funnel was charg d
with ammonium chloride (17.2 g in an aqueous solution)
and adjusted to allow dropwise addition of the ammonium
chloride to the acidic solution. When addition was
complete a heating mantle was installed and the solution
30 refluxed for one hour. At the end of this time the
dropping funnel was charged with formadehyde (85 g of a
37% aqueous solution) which was added dropwise over

a two-hour period with continued heating to maintain
reflux during th addition. After all the formaldehyde
was added, the reaction mixture was stirred under
reflux for an additional two hours, then allowed to
5   cool, yielding nitrilotrimethylenephosphonic acid (NTMP).

Example 13

         50 to 55 milligrams of NTMP prepared in Example
12 was weighed into a vial and dissolved with 0.75 ml of
distilled water. To this solution, 0.25 ml of Yb-175 in
10   dilute HCl was added. The pH of the resulting solution
was then adjusted to 10 by addition of NaOH. The solution
was then heated between 60°C and 70°C for 30 minutes to
optimize complex formation. The pH of the solution was
then adjusted to 7-8 by addition of 1 M HCl.

15   Complexes of Examples 9, 10, 11 and 13 were
tested in rats. The biodistribution data are shown
in Table II.

TABLE II

	Example Nos.			
	9	10	11	13
% Dose in Skeleton*	50 ±7	25 ±2	56 ±2	63 ±5
Blood	0.074 ±0.027	0.116 ±0.040	0.231 ±0.044	0.204 ±0.059
Liver	0.138 ±0.036	0.112 ±0.027	0.214 ±0.082	0.236 ±0.072
Bone/Blood	562 ±130	179 ±60	190 ±40	256 ±90
Bone/Muscle	619 ±192	366 ±96	572 ±214	876 ±239

Number of rats tested = 5

*Based on % dose in femur X 25

Example 14

50 to 55 milligrams of EDTMP prepared in Example 1 was weighed into a vial and dissolved with 0.75 ml of distilled water. To this solution, 0.25 ml of Lu-177 in dilute HCl was added. The pH of the resulting solution was adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C for 30 minutes to optimize complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

Example 15

55 to 60 milligrams of HEEDTMP prepared according to Example 5 was weighed into a vial and dissolved with 0.75 ml of distilled water. To this,

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0.25 ml of Lu-177 in dilute HCl was added. The pH
of the resulting solution was adjusted to 10 by addition
of NaOH. The solution was then heated between 60°C and
70°C for 30 minutes to optimize complex formation. The
5   pH of the solution was then adjusted to 7-8 by addition
of 1 M HCl.

Complexes of Examples 14 and 15 were tested
in rats. The biodistribution data are shown in Table III.

TABLE III

| | Example Nos. | |
	14	15
% Dose in Skeleton*	51 ±5	58 ±3
Blood	0.10 ±0.06	0.17 ±0.08
Liver	0.48 ±0.15	1.9 ±0.7
Kidneys	0.28 ±0.03	0.30 ±0.02
Muscle	0.44 ±0.27	0.56 ±0.16
Bone/Blood	500 ±220	370 ±320
Bone/Muscle	1380 ±1900	570 ±180

Number of rats tested = 5

* Based on % dose in femur X 25

Comparative Example

Two commercially available non-nitrogen-containing phosphonic acid compounds which are well known as diagnostically useful compounds when complexed with Tc-99m were complexed with Sm-153 and tested in the manner of the complexes of the present invention. The data comparable to that given for the examples of the invention is shown in Table A below:

TABLE A. Two Hour Biodistribution of Complexes in Rats

	^{153}Sm-MDP***	^{153}Sm-HEDP*
% Dose in Skeleton**	1.65 ±0.43	20.5 ±1.4
Blood	0.23 ±0.19	13.1 ±2.6
Liver	84.5 ±3.8	3.5 ±0.9
Kidney	0.45 ±0.19	0.99 ±0.44
Urine	0.20 ±0.14	41.3 ±4.6
Bone/Blood	8.6 ±5.0	1.3 ±0.3
Bone/Muscle	7.1 ±3.2	10.5 ±2.9

Number of rats tested = 5

* 1-hydroxyethylidine-1,1-diphosphonic acid (HEDP)
** Based on % dose in femur X 25
***Methylenediphosphonate

It should be noted that the comparative
examples in above Table A are of two non-nitrogen-
containing phosphonic acid complexes. These have
certain undesirable properties which make them inferior
to the complexes of the invention. Thus, the Sm-MDP
shows high liver uptake while the Sm-HEDP has both low
skeletal uptake and poor clearance from the blood.

Example 16

The complex tested on rats in Example 2
(Sm-153-EDTMP) was also tested in rabbits in a similar
manner. Results of biodistribution (averaged for 5
rabbits) are summarized in Table IV.

TABLE IV

% Dose in Skeleton*	66 ± 5
Blood	0.12 ± 0.10
Liver	0.95 ± 0.42
Urine	34 ± 4
Bone/Blood	900 ± 800
Bone/Muscle	1200 ± 400

* Based on % dose in femur X 20.1.

Example 17

An English Setter with a tumor in his pelvis
was injected with ~19 mCi (700 MBq) of the complex of
Example 2 (Sm-153-EDTMP). After 2 hours, the dog was
imaged using a scintillation camera. The scintillation
scan showed high uptake of the complex in the tumor and
looked very similar to an earlier scan performed using
Tc-99m-MDP. The uptake ratio of the Sm-153 complex in

the tumor to normal bone was very similar to that of the Tc-99m complex. A scintillation scan of the dog five days after treatment gave similar r sults. Seven days after the treatment the dog appeared to be in less pain as evidenced by an increase in its activity.

Example 18

A series of rats was injected with the complex of Example 2 (Sm-153-EDTMP) and sacrificed at various intervals. The rat biodistribution data is summarized in Table V. This data shows rapid biolocalization properties, e.g. bone uptake and blood clearance, as well as permanent localization of the activity in the skeletal system.

Example 19

An Irish Setter with a tumor in the femur was injected with the complex of Example 2 (Sm-153-EDTMP). The leg was amputated and samples of the tumor and normal bone were counted for Sm-153. The amount of Sm-153 in the tumor was 15-20 times that of non-lesionous bone from the same leg.

Example 20

A series of 5 rabbits was injected with the complex of Example 2 (Sm-153-EDTMP). Less than 0.15 percent of the injected dose was found in the bone marrow.

TABLE V

BIODISTRIBUTION OF Sm-153-EDTMP OVER TIME IN RATS

ORGAN	% Administered Dose After							
	15 Min.	30 Min.	1 Hour	2 Hours	5 Hours	24 Hours	48 Hours	72 Hours
Skeleton*	48 ±4	53 ±2	58 ±3	58 ±4	59 ±4	52 ±3	60 ±1	57 ±4
Blood	5.852 ±.553	2.304 ±.476	.532 ±.395	.032 ±.016	.008 ±.019	.007 ±.002	.006 ±.001	.006 ±.001
Liver	.959 ±.137	.526 ±.108	.322 ±.073	.252 ±.038	.370 ±.080	.349 ±.021	.458 ±.079	.492 ±.115
Kidneys	1.745 ±.334	.805 ±.069	.466 ±.126	.254 ±.035	.364 ±.055	.250 ±.087	.286 ±.028	.216 ±.036
Urine	28 ±3	42 ±5	47 ±3	49 ±4	46 ±3	55 ±2	50 ±2	53 ±1
Bone/Muscle	30 ±2	70 ±30	300 ±200	1500 ±500	3600 ±1900	2400 ±600	2800 ±400	3400 ±800
Bone/Blood	7 ±1	20 ±4	120 ±60	1800 ±1400	4200 ±3700	6700 ±1400	8200 ±1600	7800 ±1600

Number of rats tested = 5

*Based on % dose in femur x 25

Example 21

48 to 53 milligrams of EDTMP prepared according to Example 1 was weighed into a vial and dissolved with 0.75 ml of distilled water. To this
5   solution, 0.25 ml of Gd-159 in dilute HCl was added. The pH of the resulting solution was adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C in a water bath to optimize complex formation. The pH of the solution was then
10 adjusted to 7-8 by addition of 1 M HCl.

Example 22

55 to 60 milligrams of HEEDTMP prepared according to Example 5 was weighed into a vial and dissolved with 0.75 ml of distilled water. To this
15 solution, 0.25 ml of Gd-159 in dilute HCl was added. The pH of the resulting solution was adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C in a water bath to optimize complex formation. The pH of the solution was then adjusted to
20 7-8 by addition of 1 M HCl.

Complexes of Examples 21 and 22 were tested in rats. The biodistribution data are shown in Table VI.

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TABLE VI

| | % Dose in Example No. | |
	21	22
Skeleton*	57 ±3	60 ±4
Blood	0.15 ±0.03	0.14 ±0.02
Liver	0.25 ±0.04	0.57 ±0.07
Kidneys	0.33 ±0.09	0.58 ±0.11
Muscle	0.56 ±0.15	0.76 ±0.42
Bone/Blood	305 ±42	335 ±35
Bone/Muscle	577 ±192	548 ±419

*Calculated from % dose femur x 25

CLAIMS

1. A therapeutically effective compl x of a
paticle-emitting radionuclide which is gadolinium-159
(Gd-159), holmium-166 (Ho-166), lutetium-177 (Lu-177),
05 samarium-153 (Sm-153) or ytterbium-175 (Yb-175) with
an aminophosphonic acid derivative which is ethylene-
diaminetetramethylenephosphonic acid (EDTMP),
diethylenetriaminepentamethylenephosphonic acid
(DTPMP), hydroxyethylethylenediaminetrimethylene-
10 phosphonic acid (HEEDTMP), nitrilotrimethylene-
phosphonic acid (NTMP) or tris(2-aminoethyl)
aminehexamethylenephosphonic acid (TTHMP) or a
physiologically acceptable salt of such a complex.

2. A complex as claimed in Claim 1, characterized in
15 that said radionuclide is Gd-159, Lu-177, Sm-153 and
Yb-175.

3. A complex as claimed in Claim 2, characterized in
that said radionuclide is selected from the group
consisting of Lu-177, Sm-153 or Yb-175.

20 4. A complex as claimed in Claim 3, characterized in
that said radionuclide is Sm-153.

5. A complex as claimed in Claim 3, characterized in
that said radionuclide is Lu-177.

6. A complex as claimed in Claim 3, characterized in
25 that said radionuclide is Yb-175.

7. A compl x as claimed in Claim 2, characterized in

said radionuclide is Gd-159.

8. A complex as claimed in any one of Claims 1 to 7, characterized in that said aminophosphonic acid derivative is ethylenediaminetetramethylenephosphonic
05 acid (EDTMP).

9. A complex as claimed in any one of Claims 1 to 7, characterized in that said aminophosphonic acid derivative is diethylenetriaminepentamethylene-phosphonic acid (DTPMP).

10 10. A complex as claimed in any one of Claims 1 to 7, characterized in that said aminophosphonic acid derivative is hydroxyethylethylenediaminetrimethylene phosphonic acid (HEEDTMP).

11. A complex as claimed in any one of Claims 1 to 7,
15 characterized in that said aminophosphonic acid derivative is nitrilotrimethylenephosphonic acid (NTMP).

12. A complex as claimed in any one of Claims 1 to 7, characterized in that said aminophosphonic acid
20 derivative is tris(2-aminoethyl) aminehexamethylene-phosphonic acid (TTHMP).

13. A process for the preparation of a therapeutically effective complex as claimed in Claim 1, characterized in that a radionuclide which is
25 Gd-159, Ho-166, Lu-177, Sm-153, or Yb-175 is contacted with an aminophosphonic acid derivative which is

EDTMP, DTPMP, HEEDTMP, NTMP or TTHMP.

14. A process as claimed in Claim 13, wh r in th said contact is carried out in the presence of water at a pH of from 5 to 11.

05 15. A process as claimed in Claim 13 or Claim 14, wherein the said contact is carried out at elevated temperature.

16. A process as claimed in any one of Claims 13 to 15, including the step of adjusting the pH of the

10 complex to from 7 to 8.

17. A process as claimed in any one of Claims 13 to 16, characterized in that said radionuclide is Gd-159, Lu-177, Sm-153 or Yb-175.

18. A process as claimed in Claim 17, characterized

15 in that said radionuclide is Lu-177, Sm-153 or Yb-175.

19. A process as claimed in Clair 18, characterized in that said radionuclide is Sm-153.

20. A process as claimed in Claim 18, characterized

20 in that said radionuclide is Lu-177.

21. A process as claimed in Claim 18, characterized in that said radionuclide is Yb-175.

22. A process as claimed in Claim 17, charaterized in that said radionuclide is Gd-159.

25 23. A process as claimed in any one of Claims 13 to 22, characterized in that said aminophosphonic acid

derivativ is EDTMP.

24. A process as claimed in any on of Claims 13 to 22, characterized in that said aminophosphonic acid derivative is DTPMP.

05 25. A process as claimed in any one of Claims 13 to 22, characterized in that said aminophosphonic acid derivative is HEEDTMP.

26. A process as claimed in any one of Claims 13 to 22, characterized in that said aminophosphonic acid
10 derivative is NTMP.

27. A process as claimed in any one of Claims 13 to 22, characterized in that said aminophosphonic acid derivative is TTHMP.

28. A process as claimed in any one of Claims 13 to
15 28, characterized in that said aminophosphonic and said radionuclide acid derivative are contacted in a molar ratio of from 0.1:1 to 3000:1.

29. A complex as claimed in any one of Claims 1 to 12, for use in the treatment of cancer.

20 30. The use of a complex as claimed in any one of Claims 1 to 12, in the manufacture of a medicament for the treatment of cancer.

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